

AperTO - Archivio Istituzionale Open Access dell'Università di Torino

Degradative properties of two newly isolated strains of the ascomycetes *Fusarium oxysporum* and *Lecanicillium aphanocladii*

This is the author's manuscript

Original Citation:

Availability:

This version is available <http://hdl.handle.net/2318/1691291> since 2019-02-08T14:30:12Z

Published version:

DOI:10.1007/s10123-018-0032-z

Terms of use:

Open Access

Anyone can freely access the full text of works made available as "Open Access". Works made available under a Creative Commons license can be used according to the terms and conditions of said license. Use of all other works requires consent of the right holder (author or publisher) if not exempted from copyright protection by the applicable law.

(Article begins on next page)

1 **Degradative Properties of Two Newly Isolated Strains of the Ascomycetes**

2 ***Fusarium oxysporum* and *Lecanicillium aphanocladii***

3
4 Natalia N. Pozdnyakova¹, Giovanna C. Varese², Valeria Prigione²,

5 Ekaterina V. Dubrovskaya¹, Svetlana A. Balandina¹, Olga V. Turkovskaya¹

6 ¹*Institute of Biochemistry and Physiology of Plants and Microorganisms, Russian Academy of Sciences, Russia*

7 ²*Department of Life Sciences and Systems Biology, University of Turin, Turin, Italy*

8
9 **Corresponding author**

10 Name: Natalia Pozdnyakova

11 Postal address: IBPPM RAS, 13 Prospekt Entuziastov, Saratov 410049, Russia

12 E-mail: pozdneyakova_n@ibppm.ru Phone: +78452970403

Abstract

Two ascomycete strains were isolated from creosote-contaminated railway sleeper wood. By using a polyphasic approach combining morpho-physiological observations of colonies with molecular tools, the strains were identified as *Fusarium oxysporum* Schltdl. (IBPPM 543, MUT 4558; GenBank accession no. MG593980) and *Lecanicillium aphanocladii* Zare & W. Gams (IBPPM 542, MUT 242; GenBank accession no. MG593981). Both strains degraded hazardous pollutants, including polycyclic aromatic hydrocarbons, anthraquinone-type dyes, and oil. Oil was better degraded by *F. oxysporum*, but the aromatic compounds were better degraded by *L. aphanocladii*. With both strains, the degradation products of anthracene, phenanthrene, and fluorene were 9,10-anthraquinone, 9,10-phenanthrenequinone, and 9-fluorenone, respectively. During pollutant degradation, *F. oxysporum* and *L. aphanocladii* produced an emulsifying compound(s). Both fungi produced extracellular Mn-peroxidases, enzymes possibly involved in the fungal degradation of the pollutants. This is the first report on the ability of *L. aphanocladii* to degrade four-ring PAHs, anthraquinone-type dyes, and oil, with the simultaneous production of an extracellular Mn-peroxidase.

Keywords *Fusarium oxysporum* • *Lecanicillium aphanocladii* • degradation • pollutants • Mn-peroxidase

Introduction

Ascomycetes are a large group of higher fungi that are widely distributed in the world. They inhabit various ecological niches, are highly adaptable, and have different types of relationships with other organisms – from mutualism to parasitism. Many ascomycetes are both useful and harmful to humans, which makes them important for research and use in biotechnology. The degradative properties of ascomycetes are well-known also (Harms 2011; Aranda 2016).

Fusarium fungi are diverse and are omnipresent in nature. Except the well-known *Fusarium* pathogens, most species and strains are saprotrophic and live in soil, utilizing lignin, complex carbohydrates, and plant litter as their carbon sources. Some strains form a symbiosis with plants, protect them from diseases (Gordon et al. 1989; Lemanceau et al. 1993), and even promote host growth, increasing plant biomass and indirectly increasing the efficiency of phytoextraction (Zhang et al. 2012).

Fusarium fungi are also implicated in the degradation of pollutants. For example, *F. oxysporum* significantly reduced the concentration of oil (Fariba et al. 2010) and degraded and mineralized anthracene, phenanthrene, and pyrene (Jacques et al. 2008). Two *F. solani* strains (H30 and H50) and one *F. oxysporum*

strain (H80) converted glyphosate to unidentified metabolites (Krzysko-Lupicka and Sudol 2008). In combination with *Rhodococcus erythropolis*, *F. solani* effectively degraded benzo[*a*]pyrene, toluene, and formaldehyde (Morales et al. 2017); and in combination with *Arthrobacter oxydans* (included in microcosms with alfalfa), *F. solani* significantly reduced the concentrations of phenanthrene, pyrene, and dibenz[*a,h*]anthracene (Thion et al. 2013).

Whereas *Fusarium* fungi have been studied for more than 100 years, the genus *Lecanicillium* was introduced relatively recently (Zare and Gams 2001). The study of newly isolated *Lecanicillium* strains relates primarily to their entomopathogenic properties (Ali et al. 2017). However, some *Lecanicillium* species are biotechnologically beneficial. For example, *L. muscarium* is a potent producer of extracellular cold-resistant, chitin-hydrolyzing enzymes (Fenice 2016); and *L. aphanocladii* produces the pigments osporine, orivactaine, and dihydricricodimerol (Souza et al. 2016). *L. aphanocladii* was formerly known commonly as *Verticillium lecanii* (Zimmerman) Viegas, a producer of verticillin (Gingina et al. 1990). The degradative properties of *Lecanicillium* have been poorly studied. There are data that *L. saksenae* is a good decomposer of the pesticide pendimethalin (Pinto et al. 2012) and that *V. lecanii* is a decomposer of 2,4-dichlorophenol and 2,4-dichlorophenoxyacetic acid (Vroumsia et al. 1999) and of anthracene (Krivobok et al. 1998).

The degradative activities of Ascomycota are often attributed to their extracellular enzyme systems. These can be similar to the ligninolytic systems of basidiomycetes, which include lignin peroxidase, Mn-peroxidase, and laccase. These enzymes catalyze the key steps of the degradation of lignin and a wide range of aromatic pollutants (Wong 2009; Kadri et al. 2017). The ascomycete enzymes have received much study. *Fusarium* species have ligninolytic enzymes such as Mn-peroxidase, lignin peroxidase, and laccase, which are involved in stress reactions and in the degradation of lignocelluloses (Obruca et al. 2012). The laccases of ascomycetes have been well described. The role of laccase in fungal pathogenesis has been confirmed in numerous studies (Kwiatos et al. 2015), and the laccase genes of *F. oxysporum* were functionally analyzed (Cañero and Roncero 2008). However, no data seem available on the production of similar enzymes by *Lecanicillium*.

Thus, degradative soil-inhabiting ascomycetes that produce extracellular ligninolytic enzymes can greatly contribute to the degradation of hazardous pollutants in the environment. Searching for new species and strains of ascomycetes with degradative properties and studying their physiology, biochemistry, and ecology may promote the understanding of the role these fungi play in the self-regeneration of natural ecosystems and may promote their use in biotechnology.

The aims of this work were as follows: (1) to examine the degradative activity of two newly isolated ascomycete strains, *Fusarium oxysporum* Schltdl. (IBPPM 543, MUT 4558) and *Lecanicillium aphanocladii* Zare & W. Gams (IBPPM 542, MUT 242), toward a range of hazardous pollutants; and (2) to detect ligninolytic enzymes in these two fungi.

Materials and methods

Organism isolation and identification

The fungi had been collected from the grounds of the Saratov oil refinery. The *Fusarium* strain had been isolated from creosote-contaminated railway sleeper wood, and the *Lecanicillium* strain had been isolated from a sporocarp of *Stropharia* sp., which had been collected from oiled litter. Samples were placed in petri dishes containing an agarized basidiomycetes rich medium (Bezalel et al. 1997) with our modifications (g/L): NH_4NO_3 , 0.724; KH_2PO_4 , 1.0; $\text{MgSO}_4 \times 7\text{H}_2\text{O}$, 1.0; KCl, 0.5; yeast extract, 0.5; $\text{FeSO}_4 \times 7\text{H}_2\text{O}$, 0.001; $\text{ZnSO}_4 \times 7\text{H}_2\text{O}$, 0.0028; $\text{CaCl}_2 \times 2\text{H}_2\text{O}$, 0.033; D-glucose, 10.0; peptone, 10.0. The dishes were maintained in the dark at 24–26°C for 4–6 days. The visually clear part of the mycelium was collected and placed in new petri dishes containing the same medium.

The strains were identified as *Fusarium oxysporum* Schltdl. (IBPPM 543, MUT 4558) and *Lecanicillium aphanocladii* Zare & W. Gams (IBPPM 542, MUT 242). A polyphasic approach was used that combined morpho-physiological observations of colonies with molecular tools. DNA was extracted with a NucleoSpin kit (Macherey Nagel GmbH, Duren, DE, USA) and was amplified by PCR with specific primers for the ITS1–5.8S–ITS2 region. PCR products were purified and sequenced at the MacroGen Europe Laboratory (Amsterdam, The Netherlands). The resulting sequences were compared with the reference sequences in the online databases provided by the CBS-KNAW Collection (Westerdijk Fungal Biodiversity Institute, The Netherlands) and by the National Center for Biotechnology Information (USA). Both fungi are maintained at the IBPPM RAS Collection of Rhizospheric Microorganisms (Saratov, Russia) and at the Mycotheca Universitatis Taurinensis (Turin, Italy). The accession numbers of the sequences deposited in GenBank are MG593980 for *F. oxysporum* Schltdl. (IBPPM 543, MUT 4558) and MG593981 for *L. aphanocladii* Zare & W. Gams (IBPPM 542, MUT 242).

Examination of degradative properties

The fungi were grown submerged at 24–26°C in the basidiomycetes rich medium, or they were grown at the same temperature in Kirk's medium (Kirk et al. 1986) with our modifications (g/L): KH_2PO_4 , 0.2; $\text{MgSO}_4 \times 7\text{H}_2\text{O}$, 0.05; CaCl_2 , 0.01; thiamine, 0.0025; NH_4NO_3 , 0.724. The pH of Kirk's medium was maintained with 25 mM phosphate buffer (pH 6.0), and 1% maltose was the carbon and energy source. Ligninolytic enzyme production was increased by supplementing Kirk's medium with 0.1% Tween 80 (Jager et al. 1985). The fungi were grown in 250-mL Erlenmeyer flasks, each containing 100 mL of either basidiomycetes rich medium or Kirk's medium. Growth was in the dark at 24–26 °C for 14 days with orbital shaking (120 rpm).

Pollutants were added on day 2 of fungal growth to final concentrations of 0.05 mg/L [PAHs (anthracene, phenanthrene, fluorene, pyrene, or fluoranthene) and anthraquinone dyes (Acid Blue 62 and Reactive Blue)], and 5 g/L [oil containing alkanes (47.4%), naphthene (22.3%), low-molecular-weight aromatic hydrocarbons (4.4%), high-molecular-weight aromatic hydrocarbons (5.4%), tars (3.9%), and pyrobitumen (16.6%)]. Anthracene, fluorene, phenanthrene, pyrene, fluoranthene, Acid Blue 62, and Reactive Blue were from Fluka (Switzerland); all the other compounds were from Reachim Co. (Russia). The PAHs were added as a chloroform stock solution (5 mg per 100 µl of chloroform). The control treatments contained 100 µl of chloroform, which too was added on day 2 of fungal growth. At intervals, the mycelium was separated from the growth medium by filtration. The increase in mycelium production (mg of dry biomass) was found by weighing. Residual PAHs and oil were extracted from the bulk of the flasks (without separation of the growth medium from mycelia) with chloroform (5 mL, three times). The resulting extracts were evaporated and were analyzed as described below.

Pollutant measurements

PAHs and their metabolites were analyzed by gas chromatography with a Shimadzu 2010 instrument equipped with a flame photometric detector. Compounds were separated with an HP5 column (Agilent), and helium was the carrier gas. The column temperature was kept at 200°C for 3 min, then programmed to increase to 270°C at a rate of 15°C min⁻¹, and finally kept at 270°C for 2 min. PAH oxidation products were identified with commercial 9,10-anthraquinone, phenanthrene-9,10-quinone, and 9-fluorenone (retention times, 4.51, 5.04, and 4.33 min, respectively) as the marker compounds. The gas chromatography analysis was carried out at the Simbioz Center for the Collective Use of Research Equipment in the Field of Physical–Chemical Biology and Nanobiotechnology (Institute of Biochemistry and Physiology of Plants and Microorganisms, Russian Academy of Sciences, Saratov).

Oil disappearance was measured by adsorption chromatography with gravimetric termination. Oil was extracted three times with 5 mL of chloroform, and the extracts were evaporated and applied to a column of Al_2O_3 (10 mL). After the oil was eluted with chloroform and dried to the complete evaporation of the solvent, the amount of oil was found gravimetrically (Polunina and Kushik 1977).

Because the basidiomycetes rich medium is optically opaque, the decolorization of dyes was studied on Kirk's medium at pH 6.0. Aliquots (2 mL) were taken from the flasks at intervals, and dye disappearance was monitored spectrophotometrically by the change in the absorption spectra at 590 nm (Pozdnyakova et al. 2015).

Enzyme activity measurements

Laccase activity was measured by the oxidation rate of 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)diammonium salt (ABTS) at 436 nm ($\epsilon=29300 \text{ M}^{-1}\text{cm}^{-1}$; Niku-Paavola et al. 1988) and by the oxidation rate of syringaldazine at 525 nm ($\epsilon=65000 \text{ M}^{-1}\text{cm}^{-1}$; Leonowicz and Grzywnowicz 1981). Mn-peroxidase activity was measured by the oxidation rate of 2,6-dimethoxyphenol (DMP) with H_2O_2 and Mn^{2+} at 468 nm ($\epsilon=14800 \text{ M}^{-1}\text{cm}^{-1}$; Heinfling et al. 1998) and by the oxidation rate of 2,7-diaminofluorene (DAF) at 600 nm ($\epsilon=51000 \text{ M}^{-1}\text{cm}^{-1}$; Criquet et al. 2000) under the same conditions. Peroxidase activity was calculated as the difference between the values for DMP (or DAF) oxidation with and without H_2O_2 . Lignin peroxidase was measured by the formation of the oxidation product of veratryl alcohol at 310 nm ($\epsilon=9300 \text{ M}^{-1}\text{cm}^{-1}$; Tien and Kirk 1984). One unit of enzyme activity (U/mL) is defined as the amount of enzyme that oxidizes 1 μM of substrate per min.

Emulsifying activity measurements

The emulsifying activity of the culture medium was measured according to Cooper and Goldenberg (1987). After fungal growth (with and without the pollutants) was completed, the growth medium was mixed 2:3 with kerosene, shaken for 20 min, and left to stand at room temperature for 48 h. The emulsifying activity (E_{48}) was calculated as the ratio of emulsion volume to total liquid volume and expressed as a percentage.

Statistics

All experiments were run in triplicate, each having been repeated at least three times. Standard statistical methods were used to calculate the mean values and standard deviations (SD). Data were processed with Microsoft Excel 2003.

Results and Discussion

Ascomycetes have a strong degradative potential toward natural compounds and pollutants. *L. aphanocladii* IBPPM 542 and *F. oxysporum* IBPPM 543 were isolated from the same source (creosote-contaminated sleepers) in a search for new fungal strains with degradative properties. The isolated strains were checked for their ability to degrade PAHs from creosote used to preserve railway sleepers. PAHs are priority environmental pollutants, and their degradation and transformation are well known (Kadri et al. 2017).

In our experiments, *F. oxysporum* had weak degradative activity toward PAHs. The least recalcitrant was the three-ring fluorene, with a removal value of 42% (Fig. 1A). Possibly, the low ionization potential (7.88 eV) and the relatively high solubility (1.98 mg/L) of fluorene make it accessible for fungal degradation. A metabolite from fluorene was found whose retention time (4.33 min) corresponded to that of commercial 9-fluorenone. Phenanthrene and fluoranthene were degraded by 21% and 26%, respectively. Finally, anthracene and pyrene were not degraded by this fungus.

Data are different on the degradation of PAHs by *Fusarium* strains. The ability to degrade anthracene is species specific: for example, *F. moniliforme* degraded about 77% of anthracene from 100 mg/L, while *F. solani* did not degrade anthracene at all (Krivobok et al. 1998). In some cases anthracene cannot be a single source of carbon and energy for *Fusarium* fungi, and the need for cometabolism was often reported.

The other fungus tested was *L. aphanocladii*. The reason for our interest in it is related to its typical ecological niche: *L. aphanocladii* is usually reported to be an entomopathogenic fungus. In this work, *L. aphanocladii* was isolated only with the traditional basidiomycetes rich medium containing peptone and yeast extract; therefore, it can be considered a saprotroph.

L. aphanocladii IBPPM 542 intensely degraded all the PAHs, removing 40 to 80% for three-ring PAHs (anthracene, phenanthrene, and fluorene) and about 50% of those with four condensed rings (pyrene, fluoranthene) (Fig. 1A). Some degradation metabolites were identified. These included 9,10-anthraquinone (retention time, 4.51 min), 9,10-phenanthrenequinone (retention time, 5.04 min), and 9-fluorenone (retention time, 4.33 min), the products of degradation of anthracene, phenanthrene, and fluorene, respectively.

The appearance of the degradation products and the disappearance of the substrate were checked only after 14 days of growth. In separate experiments, in which the growth time of both fungi was extended to 28 days, the degradation products disappeared and phthalic acid was simultaneously formed (data not presented).

Oil and oil products are ubiquitous and the most abundant environmental pollutants. Undoubtedly, ascomycetes, which are a large part of the soil microbiota, participate in the degradation and detoxification of

oil hydrocarbons. Both ascomycetes used in this study were active oil degraders, with 60% of oil transformed with *L. aphanocladii* and 84% with *F. oxysporum* (Fig. 1A).

The degradation by both fungi of all pollutants used in this study was accompanied by the production of an emulsifying compound(s). Emulsifying activity of the medium (E_{48}) varied from 6.2 to 41%, depending on the fungal species and on pollutant solubility (Fig. 1B). No emulsifying activity was detected in the control (pollutant-free) treatments. The production of a biosurfactant that increases the solubility of hydrophobic compounds was found earlier in the basidiomycetes *Coriolus versicolor* and *Pleurotus ostreatus* (Arun et al. 2008; Nikiforova et al. 2009). Biosurfactant production by ascomycetes was described, too (Bhardwaj et al. 2013). However, this report is the first to describe the production of an emulsifying compound(s) by *Fusarium* and *Lecanicillium* in response to the presence of hydrophobic compounds in their growth medium.

As said above, 9,10-anthraquinone was a metabolite from PAH degradation by the fungi tested. The three-ring structure of anthraquinone occupies the centers of the molecules of synthetic anthraquinone-type dyes, which are highly resistant to biodegradation (Eichlerova et al. 2007). Synthetic dyes are also hazardous pollutants, entering the environment through runoff from paint-and-varnish and textile facilities.

Of the wide range of anthraquinone dyes available, those used most often as models are Poly R-478 and Remazol Brilliant Blue SN4R (Liu et al. 2004). Dyes of this group can be used to screen fungi for the ability to produce extracellular ligninolytic peroxidases and laccases.

In our experiments, both fungi decolorized the two anthraquinone-type dyes used, Acid Blue 62 and Reactive Blue 4. As expected, *L. aphanocladii*, an active degrader of aromatic compounds, intensely decolorized the dyes (to a level as high as 70%; Fig. 2).

Data on the decolorization activity of *F. oxysporum* and *L. aphanocladii* may indirectly point to the production of ligninolytic enzymes by these fungi. Therefore, we next measured the activities of the main ligninolytic enzymes (lignin peroxidase, Mn-peroxidase, and laccase) during pollutant degradation and in the control (pollutant-free) treatments.

In the controls, no ligninolytic enzyme activity was found. In polluted environments, both fungi produced one type of ligninolytic enzyme. The enzyme was active in the presence of H_2O_2 , and 0.5 mM Mn^{2+} produced a 2- to 3-times increase in its activity. The enzyme did not oxidize veratryl alcohol and syringaldazine, the test substrates for lignin peroxidase and laccase, respectively. From these data, the enzyme was tentatively identified as an Mn-peroxidase.

Fig. 3A1 presents the time course of Mn-peroxidase production by *F. oxysporum* (A) and *L. aphanocladii* (B) during PAH degradation. The most active inducers of *F. oxysporum* peroxidase were phenanthrene, fluoranthene, and/or their degradation products. Although anthracene and pyrene were not actively degraded by *Fusarium*, they too induced peroxidase activity (Fig. 3A1). Fig. 3A2 shows the data on polyacrylamide gel electrophoresis (PAGE) under nondenaturing conditions. The enzyme was detected only after the gels had been stained with *o*-dianizidine in the presence of H₂O₂. Throughout growth, *F. oxysporum* produced only one isoform of Mn-peroxidase, regardless of the PAH used.

L. aphanocladii, too, produced Mn-peroxidase in response to the presence of pollutants in the medium (Fig. 3B1). Best peroxidase induction was achieved with anthracene and/or its degradation products. Only one enzyme was detected by nondenaturing PAGE after the gels had been stained with *o*-dianizidine and H₂O₂ (Fig. 3B2). Throughout growth, *L. aphanocladii* produced only one isoform of Mn-peroxidase, regardless of PAH used.

For crude enzyme preparations, the fungi were grown until peroxidase production was maximal, after which the mycelium was separated from the culture medium by filtration and the culture liquid was concentrated by ultrafiltration on Amicon PM10 and was used as a source of crude peroxidases. Some catalytic properties of these peroxidases have been investigated. Both enzymes oxidized ABTS, DAF, and DMP only in the presence of H₂O₂. The reaction was largely stimulated by Mn²⁺ – by 3 and 2 times for the peroxidases of *F. oxysporum* and *L. aphanocladii*, respectively. These properties make these enzymes similar to the Mn-peroxidases of ligninolytic basidiomycetes (Wong 2009).

The Ascomycota have been mainly studied for laccase production, and several reviews have summarized laccase production and use on an industrial scale for the processing of paper pulp and for the discoloration of dyes. Ascomycete laccases have been comprehensively studied and some have been sequenced (Fernaund et al. 2006; Dekker et al. 2007; Castilho et al. 2009). *F. solani* produced three ligninolytic enzymes in the presence of different inducers (Obruca et al. 2012). Because H₂O₂ significantly increased the activities of Mn-peroxidase, lignin peroxidase, and laccase, the authors suggested that these enzymes can be involved in stress response to H₂O₂ (Obruca et al. 2012). The participation of *Fusarium* enzymes in the degradation of PAHs is still a matter of discussion. For example, Wua et al. (2010b), using *F. solani* to treat mangrove deposits contaminated by PAHs, showed that laccase is involved in the degradation of anthracene and benzo[a]anthracene, whereas lignin- and Mn- peroxidase were not detected. The laccase was isolated, purified, and characterized (Wua et al. 2010a).

In *F. oxysporum* cultures used to transform the aromatic components of the dry residue from an olive mill, Sampedro et al. (2007) detected the activities of Mn-peroxidase and Mn-independent peroxidase but did not detect laccase activity. No information on Mn-peroxidase production by *Lecanicillium* seems available.

Conclusions

Both fungi were degradative toward a range of hazardous pollutants, including PAHs, anthraquinone-type dyes, and oil. Both produced peroxidase (presumably Mn-peroxidase) and an emulsifying compound(s) in response to the presence of the pollutants in the growth medium. These properties may be necessary for the survival of the fungi in polluted environments and for the degradation of pollutants. The physiological peculiarities of *Fusarium* and *Lecanicillium*, including the functions of the peroxidases and an emulsifying compound(s), and their participation in the natural cleansing of contaminated environments will be the subject of further work.

Acknowledgements. This research was supported by a grant from the Russian Science Foundation (project no. 16-14-00081). We are grateful to Dr. M.P. Chernyshova (IBPPM RAS) for gas chromatographic analysis. We are also grateful to Dmitry N. Tychinin for his assistance in preparation of the English text of this paper.

References

- Ali S, Zhang C, Wang Z, Wang X, Wu J, Cuthbertson A, Shao Z, Qiu B (2017) Toxicological and biochemical basis of synergism between the entomopathogenic fungus *Lecanicillium muscarium* and the insecticide matrine against *Bemisia tabaci* (Gennadius). *Sci Rep* 20: 46558
- Aranda E (2016) Promising approaches towards biotransformation of polycyclic aromatic hydrocarbons with Ascomycota fungi. *Curr Opin Biotechnol* 38:1-8
- Arun A, Raja P, Arthi R, Ananthi M, Kumar K, Eyini M (2008) Polycyclic aromatic hydrocarbons (PAHs) biodegradation by basidiomycetes fungi, *Pseudomonas* isolate, and their cocultures: Comparative in vivo and in silico approach. *Appl Biochem Biotechnol* 151:132-142
- Bezalel L, Hadar Y, Cerniglia C (1997) Enzymatic mechanisms involved in phenanthrene degradation by the white rot fungus *Pleurotus ostreatus*. *Appl Environ Microbiol* 63:2495-2501

- 282 Bhardwaj G, Cameotra S, Chopra H (2013) Biosurfactant from fungi: A Review. J Pet Environ Biotechnol
283 4:doi.10.4172/2157-7463.1000160
- 284 Cañero D, Roncero M (2008) Functional analyses of laccase genes from *Fusarium oxysporum*. The American
285 Phytopathological Society. 98:509-518
- 286 Castilho F, Torres R, Barbosa A, Dekker R, Garcia J (2009) On the diversity of the laccase gene: A
287 phylogenetic perspective from *Botryosphaeria rhodina* (Ascomycota: Fungi) and other related taxa. Biochem
288 Genet 47:80-91
- 289 Cooper D, Goldenberg B (1987) Surface-active agents from two *Bacillus* species. Appl Environ Microbiol
290 53:224-229
- 291 Criquet S, Joner E, Leyval C (2001) 2,7-Diaminofluorene is a sensitive substrate for detection and
292 characterization of plant root peroxidase activities. Plant Science 161:1063-1066
- 293 Dekker R, Barbosa A, Giese E, Godoy S, Covizzi L (2007) Influence of nutrients on enhancing laccase
294 production by *Botryosphaeria rhodina* MAMB-05. Int Microbiol 10:177-185
- 295 Eichlerova I, Homolka L, Benada O, Kofronova O, Hubalek T, Nerud F (2007) Decolorization of Orange G and
296 Remazol Brilliant Blue R by the white rot fungus *Dichomitus squalens*: Toxicological evaluation and
297 morphological study. Chemosphere 69:795-802
- 298 Fariba M, Simin N, Alireza M, Ramin N, Doustmorad Z, Gholam K, Abdolkarim C (2010) Phytoremediation of
299 petroleum-polluted soils: Application of *Polygonum aviculare* and its root-associated (penetrated) fungal
300 strains for bioremediation of petroleum-polluted soils. Ecotoxicol Environ Safety 73:613-619
- 301 Fenice M (2016) The psychrotolerant antarctic fungus *Lecanicillium muscarium* CCFEE 5003: A powerful
302 producer of cold-tolerant chitinolytic enzymes. Molecules 21:447
- 303 Fernaud J, Marina A, González K, Vázquez J, Falcón M (2006) Production, partial characterization and mass
304 spectrometric studies of the extracellular laccase activity from *Fusarium proliferatum*. Appl Microbiol
305 Biotechnol 70:212-221
- 306 Gingina GM, Mitina GV, Pavlushin VA (1990) Toxigenicity of *Verticillium lecanii* (Zimmermann) viegas
307 natural isolates. Mycology and Phytopathology 24:576-582 (in Russian)
- 308 Gordon T, Okamoto D, Jacobson D (1989) Colonization of muskmelon and non-susceptible crops by *Fusarium*
309 *oxysporum* F. sp. melonis and other species of *Fusarium*. Phytopathology 79:1095-1100
- 310 Harms H (2011) Untapped potential: exploiting fungi in bioremediation of hazardous chemicals. Nature
311 Reviews. Microbiology 9:177-192

- Heinfling A, Martinez M, Martinez A, Bergbauer M, Szewzyk U (1998) Purification and characterization of peroxidases from dye-decolorizing fungus *Bjerkandera adusta*. FEMS Microbiol Lett 165:43-50
- Jacques R, Okeke B, Bento F, Teixeira A, Peralba M, Camargo F (2008) Microbial consortium bioaugmentation of a polycyclic aromatic hydrocarbons contaminated soil. Bioresour Technol 99:2637-2643
- Jager A, Croan S, Kirk T (1985) Production of ligninases and degradation of lignin in agitated submerged cultures of *Phanerochaete chrysosporium*. Appl Environ Microbiol 50:1274-1278
- Kadri T, Rouissi T, Brar SK, Cledon M, Sarma S, Verma M (2017) Biodegradation of polycyclic aromatic hydrocarbons (PAHs) by fungal enzymes: A review. J Environ Sci 51:52-74
- Kirk T, Croan S, Tien M, Murtagh K, Farrell R (1986) Production of multiple ligninases by *Phanerochaete chrysosporium* effect of selected growth condition and use mutant strain. Enzyme Microbial Technol 8:27-32
- Krivobok S, Miriouchkine E, Seigle-Murandi F, Benoit-Guyod J-L (1998) Biodegradation of anthracene by soil fungi. Chemosphere 37:523-530
- Krzysko-Lupicka T, Sudol T (2008) Interactions between glyphosate and autochthonous soil fungi surviving in aqueous solution of glyphosate. Chemosphere 71:1386-1391
- Kwiatos N, Ryngajłło M, Bielecki S (2015) Diversity of laccase-coding genes in *Fusarium oxysporum* genomes. Frontiers in Microbiology 6:933
- Lemanceau P, Bakker P, DeKogel W, Alabouvette C, Schippers B (1993) Antagonistic effect of nonpathogenic *Fusarium oxysporum* Fo47 and pseudobactin 358 upon pathogen *Fusarium oxysporum* F. sp. Dianthi. Appl Environ Microbiol 59:74-82
- Leonowicz A, Grzywnowicz K (1981) Quantitative estimation of laccase forms in some white-rot fungi using syringaldazine as a substrate. Enzyme Microbial Technol 3:55-58
- Liu W, Chao Y, Yang X, Bao H, Qian S (2004) Biodecolourization of azo, anthraquinonic and triphenylmethane dyes by white rot fungi and laccase-secreting engineered strain. J Ind Microbiol Biotechnol 31:127-132
- Morales P, Cáceres M, Scott F, Díaz-Robles L, Aroca G, Vergara-Fernández A (2017) Biodegradation of benzo[α]pyrene, toluene, and formaldehyde from the gas phase by a consortium of *Rhodococcus erythropolis* and *Fusarium solani*. Appl Microbiol Biotechnol 101:6765-6777
- Nikiforova SV, Pozdnyakova NN, Turkovskaya OV (2009) Emulsifying agent production during PAHs degradation by the white rot fungus *Pleurotus ostreatus* D1. Curr Microbiol 58:554-558

- 341 Niku-Paavola M, Karhunen E, Salola P, Raunio V (1988) Ligninolytic enzymes of the white rot fungus *Phlebia*
 342 *radiata*. Biochem J 254:877-884
- 343 Obruca S, Marova I, Matouskova P, Haronikova A, Lichnova A (2012) Production of lignocellulose-degrading
 344 enzymes employing *Fusarium solani* F-552. Folia Microbiol 57:221-227
- 345 Pinto A, Serrano C, Pires T, Mestrinho E, Dias L, Teixeira D, Caldeira A (2012) Degradation of terbuthylazine,
 346 difenoconazole and pendimethalin pesticides by selected fungi cultures. Sci Total Environ 435-436:402-410
- 347 Polunina AG, Kushik GI (1977) Metody analiza organicheskogo veshchestva porod, nefi i gaza (Methods of
 348 Analyslis of Organic Matter in Rocks, Oil, and Gas). In: Ryl'kov AV (ed) Tyumen': Tr. Zap.-Sib. NIGNI,
 349 122 (in Russian).
- 350 Pozdnyakova NN, Jarosz-Wilkolazka A, Polak J, Graz M, Turkovskaya OV (2015) Decolourisation of
 351 anthraquinone-and anthracene-type dyes by versatile peroxidases from *Bjerkandera fumosa* and *Pleurotus*
 352 *ostreatus* D1. Biocatal Biotransform 33:69-80
- 353 Sampedro I, D'Annibale A, Ocampo J, Stazi S, García-Romera I (2007) Solid-state cultures of *Fusarium*
 354 *oxysporum* transform aromatic components of olive-mill dry residue and reduce its phytotoxicity.
 355 Bioresource Technol 98:3547-3555
- 356 Souza P, Grigoletto T, de Moraes L, Abreu L, Guimarães L, Santos C, Galvão L, Cardoso P (2016)
 357 Production and chemical characterization of pigments in filamentous fungi. Microbiology 162:12-22
- 358 Thion C, Cebon A, Beguiristain T, Leyval C (2013) Inoculation of PAH-degrading strains of *Fusarium solani*
 359 and *Arthrobacter oxydans* in rhizospheric sand and soil microcosms: microbial interactions and PAH
 360 dissipation. Biodegradation 24:569-581
- 361 Tien M, Kirk K (1984) Lignin-degrading enzyme from *Phanerochaete chrysosporium*: purification,
 362 characterization, and catalytic properties of a unique H₂O₂-requiring oxygenase. Proc Nat Acad Sci USA
 363 81:2280-2284
- 364 Vroumsia T, Steiman R, Seigle-Murandi F, Benoit-Guyod J-L (1999) Effects of culture parameters on the
 365 degradation of 2,4-dichlorophenoxyacetic acid (2,4-D) and 2,4-dichlorophenol (2,4-DCP) by selected fungi.
 366 Chemosphere 39:1397-1405
- 367 Wong DWS (2009) Structure and action mechanism of ligninolytic enzymes. Appl Biochem Biotechnol
 368 157:174-209
- 369 Wua Y-R, Luo Z-H, Chow R, Vrijmoed L (2010a) Purification and characterization of an extracellular laccase
 370 from the anthracene-degrading fungus *Fusarium solani* MAS2. Bioresource Technol 101:9772-9777

371 Wua Y-R, Luo Z-H, Vrijmoed L (2010b) Biodegradation of anthracene and benzo[a]anthracene by two
372 *Fusarium solani* strains isolated from mangrove sediments. Bioresource Technol 101:9666-9672

373 Zare R, Gams W (2001) A revision of *Verticillium* section Prostrata. IV. The genera *Lecanicillium* and
374 *Simplicillium* gen. nov. Nova Hedwig 73:1-50

375 Zhang X, Lin L, Chen M, Zhu Z, Yang W, Chen B, Yang X, An Q (2012) A nonpathogenic *Fusarium*
376 *oxysporum* strain enhances phytoextraction of heavy metals by the hyperaccumulator *Sedum alfredii* Hance.
377 J Hazard Mater 229-230:361-370

378

379

380

381

382

383

384

385

386

387

388

389

390

391

392

393

394

395

396

397

398

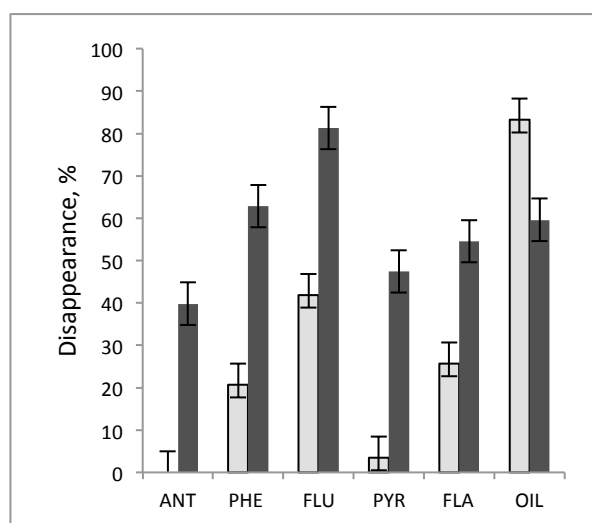
399

400

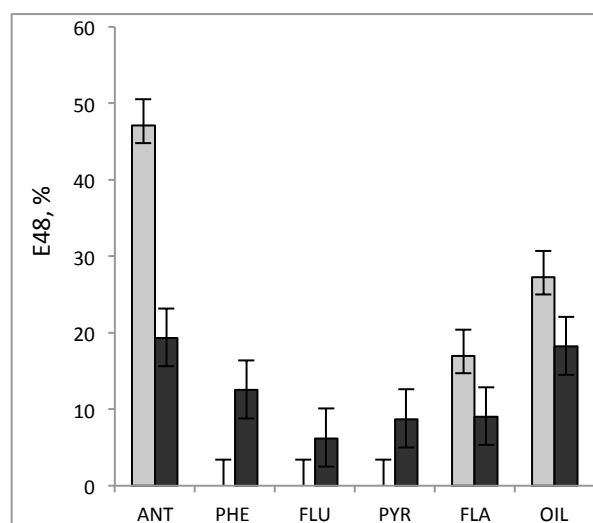
Table 1 Catalytic properties of *F. oxysporum* and *L. aphanocladii* peroxidases

Fungus	Activity, U/mL					
	-Mn ²⁺			+Mn ²⁺		
	ABTS	DMP	DAF	ABTS	DMP	DAF
<i>F. oxysporum</i>	2.8±0.7	4.1±0.5	3.6±0.4	9.5±1.6	14.0±2.4	12.2±0.8
<i>L. aphanocladii</i>	2.4±0.8	5.3±0.8	4.8±0.6	5.5±1.2	12.4±1.8	11.0±0.9

A



B



422 **Fig.1** Pollutant disappearance (A) and emulsifying activity production (B) during submerged cultivation of *F.*
 423 *oxysporum* (□) and *L. aphanocladii* (■): ANT, anthracene; PHE, phenanthrene; FLU, fluorene; PYR, pyrene;
 424 FLA, fluoranthene. Data always represent means \pm standard deviations from triplicate cultures, $P \leq 0.05$.

425

426

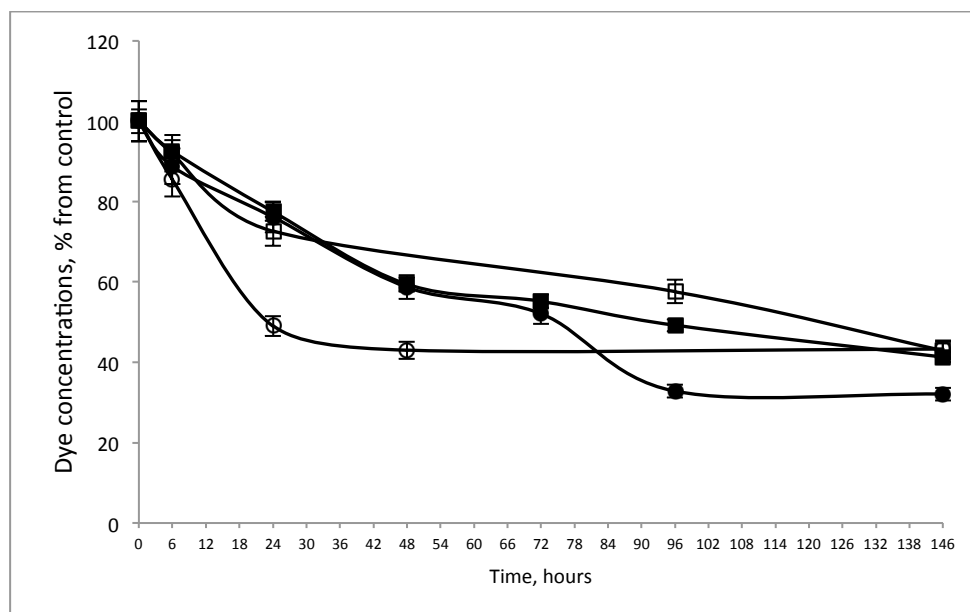


Fig. 2 Decolorization of anthraquinone-type dyes by *F. oxysporum* (■, □) and *L. aphanocladii* (●○): Acid Blue 62 (■, ●) and Reactive Blue 4 (□, ○). Data always represent means \pm standard deviations from triplicate cultures, $P \leq 0.05$.

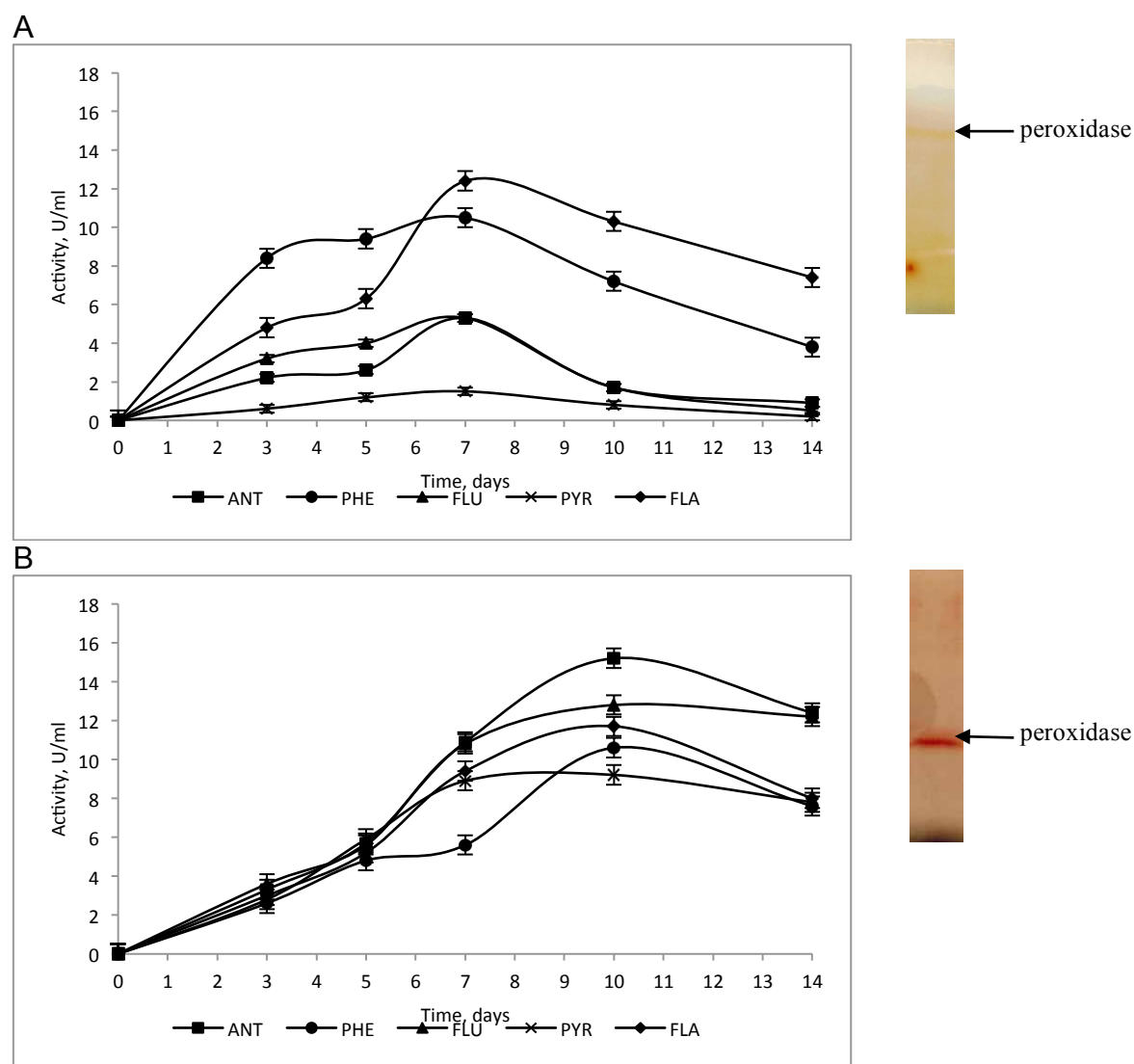


Fig. 3 Time course of Mn-peroxidase production by *F. oxysporum* (A1) and *L. aphanocladii* (B1) and nondenaturing PAGE of the detected enzymes (A2, B2). Data always represent means \pm standard deviations from triplicate cultures, $P \leq 0.05$.